Remarks

Please cancel claim 94, without prejudice. Claim 94 having been canceled herewith, claims 1-13 are pending and under examination. Reconsideration and withdrawal of the rejections of claims 1-13 are respectfully requested.

Rejection under 35 U.S.C. §103

The Examiner rejected claims 1-13 under 35 U.S.C. §103(a) as being unpatentable over Madison et al. (*Microbiol. Mol. Biol. Rev.*, 1999; *63*(1):21-53) in view of Clemente et al. (U.S. Patent No. 5,849,894) and Lee et al. (*Int. J. Biol. Macromol.*, 1999; *25*(1-3):31-36). This rejection is respectfully traversed.

In order to establish a *prima facie* case of obviousness, the Examiner must establish that there is a motivation to combine the documents (or modify the teachings of a document) to achieve the claimed invention, with a reasonable expectation of success. Further, the references must teach or suggest every element of the claimed invention. For at least the reasons set forth below, it is respectfully submitted that the Examiner has failed to make the requisite showing of a *prima facie* case of obviousness.

Madison et al. is a review article generally describing metabolic engineering of poly(3-hydroxyalkanoates) (PHAs). At page 44 the authors describe accumulation of P(3HB) by *Saccharomyces cerevisiae* cells when just the P(3HB) polymerase gene from *R. eutropha* was introduced into the cells. Madison et al. note the low level of accumulation of P(3HB) in Leaf et al., and suggest that elevation of thiolase and reductase activities may lead to improved P(3HB) production in yeast.

The Examiner asserts that the difference between Madison et al. and the instant invention is that Madison et al. "does not teach a method of producing PHA using a transgenic yeast comprising all three heterologous [genes] in a single nucleic acid construct."

Applicants disagree, and submit that there are a number of additional differences between the claimed invention and the cited art. Further, for the reasons that follow, it is respectfully

submitted that in making the comparison the Examiner has inadvertently mischaracterized the invention.

Claim 1 presently under examination reads as follows:

1. A method for the production of a polyhydroxyalkanoate (PHA) comprising:

providing a transgenic yeast cell comprising a first nucleic acid fragment comprising a heterologous nucleotide sequence encoding a PHA polymerase and at least one second nucleic acid fragment comprising a heterologous nucleotide sequence selected from the group consisting of a heterologous nucleotide sequence encoding an acetoacetyl-CoA reductase and a heterologous nucleotide sequence encoding a β-ketothiolase;

culturing the transgenic yeast cell under anaerobic conditions to cause the production of PHA; and

isolating the PHA from the yeast cell.

Applicants note that claim 1 recites at least two nucleic acid fragment, not three, and does not stipulate that they must be present on a single nucleic acid construct. Moreover, claim 1 recites culturing the transgenic yeast cell under anaerobic conditions, which is also not taught in Madison et al., as detailed further below.

Clemente et al. is cited by the Examiner as teaching a method of expressing three genes via a single nucleic acid construct. However, Applicants point out that the teachings of Clemente et al. are limited to bacteria and plants ("PHA synthesis in bacteria and plants requires at least three genes: β-ketothiolase (phaA), acetoacetyl-CoA reductase (phaB), and PHA synthase (phbC)" Clemente et al. at col. 15, lines 10-12). Indeed, while Clemente et al. contains an extensive (and prophetic, it should be added) discussion of PHA synthesis in bacteria and plants using the *R. rubrum* PHA synthase (see Clemente et al., Example 6), mention of yeast as a host organism is conspicuously absent.

Madison et al. is itself silent as to the culture conditions (aerobic vs. anaerobic) for production of PHB in yeast.. However, the *S. cerevisiae* experiments that are reported in Madison et al. (i.e., citing Leaf et al., Microbiology, 1996, 142:1169-1180) were conducted under *aerobic* conditions (Leaf et al. at page 1170: "Dissolved oxygen did not drop below 90% over the course of batch growth."). Although low yield is noted, neither Madison et al. nor the cited reference Leaf et al. suggest the use of anaerobic growth conditions to improve it.

The Examiner states that "production of polyhydroxyalkanoates using anaerobic/fermentation methods are well known and performed in the art" citing Lee et al. and Clemente et al. In response, Applicants respectfully submit that the Examiner has erred in equating "fermentation" with "anaerobic culture conditions." The word "fermentation" as used in industrial microbiology does not necessarily imply *anaerobic* culture conditions. The term fermentation is commonly used to simply describe bacterial cultures, especially large scale cultures, regardless of the oxygen levels in the culture. Indeed, the Oxford Dictionary of Biochemistry and Molecular Biology (Oxford University Press, New York, 1997, p. 239), in addition to giving a first definition of fermentation that describes anaerobic breakdown of glucose to lactate or ethanol, provides as its second definition for fermentation:

> 2 (*in biotechnology*) the use of microorganisms or cultured cells to produce useful materials, such as antibiotics, beverages, enzymes, and some commodity chemicals.

See also the Frequently Asked Questions from the website of Metabolix, Inc., submitted herewith, which describes their preferred process of "aerobic fermentation" or "aerated fermentation" to produce PHAs.

With respect to Clemente et al., "fermentation [of *R. rubrum*] with various carbon sources" is taught at col. 4, lines 63-65. However, in addition to the absence of any indication whether this fermentation occurred under aerobic or anaerobic conditions, this teaching is limited to the context of *endogenous* production of PHA in *R. rubrum* (see col. 4, lines 62-65). The only actual recitation of "anaerobic" culture conditions in Clemente et al. appears at col. 5,

lines 42-43, describing the growth and harvest of bacteria and not the production of PHA. Anaerobic culture conditions are neither taught nor suggested in Example 6 of Clemente et al. for metabolically engineered bacterial or plant cells expected to produce PHA, much less for yeast cells which are not even mentioned in Clemente et al.

Like Clemente et al., Lee et al. also uses the word "fermentation" in the context of industrial microbiology, and there is no reason to assume that anaerobic fermentations are necessarily implied by the use of that term without further qualification. Lee et al. do describe production of P(3HB) in *E. coli* cultures using "insufficient oxygen" (1-3% of air saturation), i.e. oxygen stress, during both the active cell growth phase and the active P(3HB) synthesis phase (Lee te al., page 33). However Lee et al. found that insufficient oxygen hampered cell growth in the active cell growth phase, and did not enhance P(3HB) during either the active cell growth phase or the active P(3HB) synthesis phase. Thus Lee et al. fail to provide a motivation to combine the cited references.

Even if the cited references could be construed to suggest that anaerobic culture conditions be used to produce PHA in transgenic yeast, Applicants respectfully submit that the combination of Madison et al., Clemente et al. and/or Lee et al. does not provide a reasonable expectation of success for achieving production of PHA in transgenic yeast under anaerobic conditions. The "fermentation" references cited by the Examiner (namely, Clemente et al. and Lee et al.) are directed to bacterial fermentations, not yeast. Yeast and bacteria are substantially different organisms, one being prokaryotic while the other is eukaryotic; they require substantially different handling; and they have significantly different metabolisms. Anaerobic production of PHA is particularly complex, making techniques useful in one cell type difficult to apply to another cell type. As *E. coli* and *S. cerevisiae* are substantially different, both in terms of their culturing and their metabolism, and as anaerobic culturing provides further complications, there is no reasonable expectation of success based upon the cited references that PHA could be produced anaerobically in yeast.

Furthermore, it is respectfully submitted that the Examiner has essentially already appreciated the substantial differences between production of PHA in bacteria and the

production of PHA in yeast by restricting methods in yeast from methods in bacterial cells. Specifically, the claims of the present application were earlier subject to a restriction requirement, in which claims 1-13 and 27-33 (Group I, to a method of producing PHA in transgenic *yeast*) were restricted from claims 62-67 and 78-83 (Group VI, to a method of producing PHA in transgenic *bacterial* cells) (emphasis added). The Examiner, on p. 4 of the restriction requirement, stated that "[t]he methods of inventions I-VIII are patentably distinct as directed to materially different methods employing different products. Inventions I-VIII are also patentably distinct from each other because the methods have different effects and utilities." As stated in M.P.E.P. § 802.01, "[t]he term "distinct" means that two or more subjects as disclosed are related ... but are capable of separate manufacture, use, or sale as claimed, AND ARE PATENTABLE (novel and unobvious) OVER EACH OTHER (though they may each be unpatentable because of the prior art)" (emphasis in the original). Thus, the Examiner's statement in the Office Action at p. 4-5, that "[a]lthough Lee et al. uses bacteria, one of ordinary skill in the art can apply similar methodology in producing polyhydroxyalkanoates using yeast in anaerobic conditions" is contradicted by the restriction earlier applied to the present application, in which anaerobic production of PHA was restricted between yeast and bacteria.

For at least the reasons provided above, reconsideration and withdrawal of the rejection of claims 1-13 under 35 U.S.C. §103(a) as being unpatentable over Madison et al. in view of Clemente et al. and Lee et al. is respectfully requested.

Summary

It is respectfully submitted that the pending claims 1-13 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number if it is believed that prosecution of this application may be in any way expedited or assisted thereby.

Respectfully submitted for
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CERTIFICATE UNDER 37 CFR §1.8:
The undersigned hereby certifies that the Transmittal Letter and the paper(s), as described hereinabove, are being transmitted by facsimile in accordance with 37 CFR §1.6(d) to the Patent and Trademark Office, addressed to Commissioner for Patents, Mail Stop Amendment, P.O. Box 1450, Alexandria, VA 22313-1450, on this 10th day of January, 2005, at 1:00pm (Central Time).

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